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All Roads Lead to Neuroscience: The 2013 Nobel Prize in Physiology or Medicine

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Numerous metaphors have been employed to describe the achievements of the 2013 Nobel Laureates in Physiology or Medicine, James E. Rothman, Randy W. Schekman, and Thomas C. Südhof, who were honored for "their discoveries of machinery regulating vesicle traffic, a major transport system in our cells." Most of these metaphors referred to the mundane issue of business logistics, and there is probably no other cell type in which the logistics problem is more pressing than in neurons.

Manufacturing companies have to get their products in the right quantity at the right time in the right condition to the right customer at the right location. Eukaryotic cells have a similar logistics problem. They produce thousands of proteins that have to be trafficked accurately to many different subcellular compartments. The 2013 Nobel Laureates in Physiology or Medicine deciphered the molecular mechanisms of the vesicle-based secretory pathway that controls the fate of proteins from their production site to their specific target location and showed how the secretory machinery is "honed" in nerve cells for fast neurotransmitter release at synapses.

The Neuronal Likeness of Yeast Cells

The budding yeast *Saccharomyces cerevisiae* is the genetically and physiologically best-studied eukaryotic cell, and no neuroscientist with sufficient cell biological knowledge would question the importance that yeast models have had for modern neurobiology. Even clinically focused neuroscientists have started to use yeast cells to analyze pathomechanisms and develop therapies, for example, in the context of neurodegenerative diseases. However, in 1976, when Randy W. Schekman chose *Saccharomyces cerevisiae* as a model to study cell growth and secretion, it was not clear whether yeast cells employ a proper secretory pathway, let alone whether any such yeast pathway would be relevant for human cells—or neurons.

In his Nobel Lecture at the Karolinska Institute, Schekman emphasized that his choice of topic and model system was mainly influenced by George E. Palade's description of protein synthesis, transport, and secretion in exocrine pancreatic cells, which led to the Nobel Prize in 1974, and by Leland H. Hartwell's success in using yeast genetics to decipher the mechanisms that drive the cell-division cycle, which was honored with the 2001 Nobel Prize. Inspired by these pioneers, Schekman's laboratory set out to systematically identify yeast mutants with aberrant cell-surface growth. Assuming that such mutants would be extremely sick, the focus was on temperature-sensitive mutants, which turned out to be crucial for the success of the endeavor.

The initial work of Schekman led to the identification of mutations in 23 so-called *sec* genes, which caused the accumulation of vesicles and other organelles in mutant cells (Novick and Schekman, 1979; Novick et al., 1980). The finding that certain *sec* mutants accumulate vesicles in defined subcellular compartments represented the first direct demonstration of a role of transport vesicles in protein and membrane trafficking through the secretory pathway, a notion that had been posed earlier by Palade. Subsequently, Schekman and several other yeast geneticists identified a total of some 100 genes with related functions. This led ultimately—as Schekman pointed out in his Nobel Lecture—to a detailed definition of the "genetic contour of the secretory pathway," with individual genes mapped to defined steps in the pathway from protein translocation into the endoplasmic reticulum, via -endoplasmic-reticulum-to-Golgi transport, Golgi maintenance, and Golgi sorting, to vesicle fusion (Figure 1). In follow-up studies, Schekman and colleagues cloned the *sec* genes and used biochemical and cell biological assays to study the functions of the corresponding SEC proteins. This work led to several seminal discoveries, for example, of the role of the COP-II vesicle coat in endoplasmic-reticulum-to-Golgi transport (Barlowe et al., 1994).

Schekman ended his Nobel Lecture with an excursus into human pathophysiology, describing how mutations in proteins involved in COP-II coat formation lead to diseases, such as cranio-lenticulo-sutural dysplasia in the case of mutations of SEC23A. This justification of the general relevance of his work may have been necessary to impress all the modern skeptics, who believe that molecules and molecular mechanisms do not matter much—particularly not in understanding brain function. But as regards scientists with even only a tad bit of molecular and cellular inclination, Schekman was preaching to the converted.

His pioneering work did not only crucially influence the studies of his 2013 colaureates James E. Rothman and Thomas C. Südhof but has deeply penetrated and fostered all of cell biology, particularly the field of molecular and cellular neuroscience. About a third of all proteins encoded by the human genome require transport through the secretory pathway, whose genetic and molecular outline Schekman's work has defined. The release of growth factors, the plasma membrane exposure of their receptors, the transport of ion channels to the cell

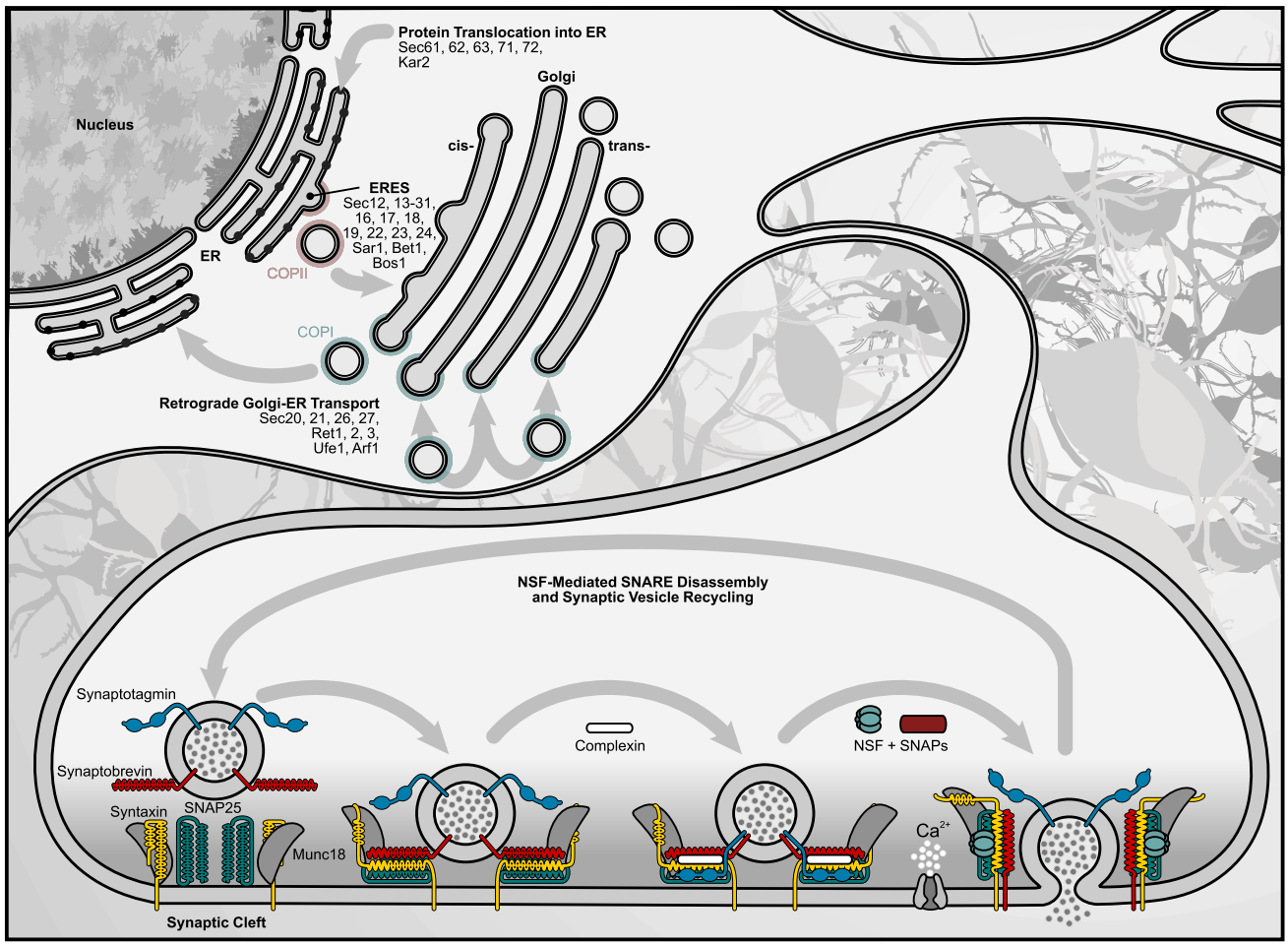

Figure 1. The Nobel Prize in a Nutshell
The secretory pathway and the synaptic vesicle fusion machinery. ER, endoplasmic reticulum; ERES, ER exit site. See text for details.

surface, the release of hormones and neurotransmitters, the dynamic cell surface exposure of neurotransmitter receptors ... every aspect of brain cell biology, and, consequently, of brain function depends on the secretory system. It is therefore not at all surprising that the genes discovered by Schekman are critical for human health and have featured very prominently in neurobiology. For instance, mammalian SEC1 homologs of the Munc18 family are essential regulators of neurotransmitter release and several other cellular processes, and mammalian SEC4 homologs of the Rab family of small GTPases regulate multiple membrane and protein trafficking steps in neurons, including transmitter release and receptor recycling. The list of examples could be continued endlessly as there are over 100 SEC and SEC-related proteins. It ends here because I, at least, have been a "yeast believer" for decades.

A Biochemist's Journey to Neuroscience

James E. Rothman's "maiden voyage" into the field of secretory trafficking started at almost the same time as Schekman's but followed a strikingly different course. Trained as a physicist, Rothman initially applied to do graduate work in the neurosciences but was rejected, as he stated in his Nobel Lecture. Thus "coerced" to become a biochemist instead, Rothman continued the legacy of Palade, one of his main inspirators, by applying brute-force biochemistry.

At the time, Palade had put forward the notion that transport vesicles are the vehicles by which membranes and proteins are trafficked through the secretory pathway. However, it was unclear as to how the corresponding cellular logistics problem is solved, that is, how the right cargo is delivered to the right place at the right time. Rather than pursuing the then prevalent idea that the cellular microanatomy defines the specificity of cellular trafficking, Rothman based his work on the hypothesis that there must be an intrinsic molecular specificity by which transport vesicles recognize their correct target membranes.

To test this hypothesis and search for proteins that define the specificity of vesicular trafficking, Rothman chose an equally daring and ingenious cell-free assay system that allowed him to follow the maturation of the glycoprotein VSVG as it is trafficked through the different Golgi compartments (Balch et al., 1984). Using this assay, Rothman's group

identified the role of the vesicular COP-I coat in intra-Golgi trafficking (Malhotra et al., 1989), showed that the enzyme NSF is required for the intra-Golgi transport process and involved in regulating vesicle fusion (Block et al., 1988), and found that NSF requires soluble membrane attachment proteins, called SNAPs (Clary et al., 1990) (Figure 1). Subsequent work showed that NSF and SNAPs are the mammalian homologs of yeast SEC18 and SEC17, respectively. This fascinating convergence of Schekman's genetic and Rothman's biochemical studies provided a crucial cross-validation and showed that the two pioneers were up to something of very general relevance.

But Rothman's initial discoveries did not solve the cellular logistics problem. NSF and SNAPs are soluble and additional work had indicated that they might be involved in several different membrane trafficking and fusion events. They could not possibly be responsible for the specificity of vesicular fusion reactions, they had to have target proteins in membranes that provide specificity and possibly even execute fusion. In search for these targets, Rothman turned to the brain, where NSF and SNAPs are most abundant. Using recombinant NSF/SNAP complexes as an affinity matrix, his group purified three proteins that bound to NSF/SNAP complexes and eluted from them upon ATP hydrolysis: the synaptic vesicle protein VAMP/Synaptobrevin, the presynaptic plasma membrane protein Syntaxin, and the synaptosomal protein SNAP25 (Söllner et al., 1993a), which Rothman collectively termed SNAP receptors or SNAREs. At first sight, the corresponding protein sequencing data must have caused a collective transient cardiac arrest in the Rothman lab, as two of the three proteins were already well-known suspects. VAMP/Synaptobrevin had just been shown to be a proteolytic substrate of certain clostridial neurotoxins that block neurotransmitter release, indicating a key role in synaptic vesicle fusion (Schiavo et al., 1992), and Syntaxin had been discovered previously as a binding partner of the synaptic vesicle protein Synaptotagmin, indicating a role in synaptic vesicle docking to the plasma membrane (Bennett et al., 1992).

The discovery of the SNARE proteins as targets of the NSF/SNAP complex provided the key insight into the mechanism of vesicle fusion, but it took quite some time to sort out the facts. Indeed, the initial SNARE discovery was followed by substantial controversy in the field, with multiple and often diametrically opposed models being propagated, e.g., regarding the role of NSF and the SNAPs in the actual fusion reaction, or the orientation of the SNARE proteins in the complex. In the introduction to Rothman's Nobel Lecture by the Nobel Assembly, this phase of model development was likened to the maturation of a good red wine, which needs time to develop its full body. Ultimately, the discoveries that VAMP/Synaptobrevin, Syntaxin, and SNAP25 form a tight complex that is dissociated by NSF (Söllner et al., 1993b), that SEC18/SEC17 (i.e., NSF/SNAP) action can precede membrane docking and fusion (Mayer et al., 1996), that the three SNARE proteins alone are sufficient to execute membrane fusion in vitro (Weber et al., 1998), and that the SNARE complex is formed by the parallel association of helical SNARE motifs within the three SNARE proteins (Sutton et al., 1998) settled the case: one SNARE motif each of VAMP/Synaptobrevin and Syntaxin and two SNARE motifs of SNAP25 zipper up to form a four helix bundle and thereby drive the membrane fusion reaction, whereas the NSF/SNAP complex is responsible for dissociating SNARE complexes, e.g., after fusion (Figure 1). Subsequent studies by Rothman's group and several other laboratories identified large families of VAMP/Synaptobrevin-, Syntaxin-, and SNAP25-like proteins with different subcellular distributions and showed that only a subset of SNARE protein combinations lead to SNARE complex formation and membrane fusion. This work demonstrated that SNARE proteins do not only execute the fusion reaction but also contribute substantially to the specificity of vesicle fusion reactions, thereby helping to deal with the notorious logistics problem in the secretory pathway.

Rothman's Nobel Lecture culminated with a discussion of synaptic vesicle fusion, whose speed and temporal accuracy continue to pose a problem for the SNARE model of membrane fusion. SNARE-mediated fusion in vitro is rather slow and asynchronous, as is the case with many cellular vesicle fusion reactions. In contrast, neurotransmitter secretion from synaptic vesicles occurs with millisecond precision and is tightly controlled by the intracellular Ca^{2+} concentration, which can boost the synaptic vesicle fusion rate by up to one million times. This discrepancy led to the notion that SNARE-mediated synaptic vesicle fusion must be controlled by a specialized protein machinery that keeps some synaptic vesicles in a fusion-ready—or primed—state at any given time, that prevents or "clamps" unwanted spontaneous fusion prior to a stimulus, and that boosts the somewhat sluggish SNARE machinery upon action potential induced Ca^{2+} influx. Using in vitro fusion assays, Rothman and his colleagues found that Complexin, a protein that had previously been shown to bind to assembled SNARE complexes (McMahon et al., 1995) and to be required for normal synaptic transmitter release (Reim et al., 2001), can indeed "clamp" SNARE-mediated fusion (Giraudo et al., 2006). While still controversial in the field of molecular and cellular neuroscience, his "clamp" model of Complexin function has made Rothman a neuroscientist after all, and countless neuroscience colleagues, including myself, have been deeply inspired by his discoveries—and by his visionary and often daring hypotheses.

A Paradigma of Molecular Neuroscience

At the time of the SNARE complex discovery, Thomas C. Südhof had already been well on his way to a systematic molecular cartography of presynaptic function. Südhof's choice of his research topic was based on his fascination with synapses, the "fundamental information processors" in the brain, as he put it in his Nobel Lecture. This fascination with synapses had been triggered during Südhof's graduate work with Victor Whittaker and by the studies of Bernard Katz, Ulf von Euler, and Julius Axelrod, who won the Nobel Prize in 1970—and it was fortunately not lost during a subsequent "detour" into cholesterol biology.

I vividly remember my first encounter with Südhof in 1987, a year after he had

started his own laboratory. At the time, I was a young PhD student with Reinhard Jahn, who had invited Südhof to discuss what would later become an extremely fruitful collaboration. The two decided no less than to embark on a joint project to decipher the molecular mechanisms of synaptic transmitter release. "Yeah, sure!" I thought, very skeptically, "Good luck!" I was of course aware of the importance of neurotransmitter release. I knew that transmitters are released from synaptic vesicles with extreme speed and precision and that the release is triggered by Ca^{2+} ions in a highly cooperative manner—but not a single molecular component of the synaptic release machinery had been discovered at the time.

Neuron Editorial Board member Thomas C. Südhof with his wife Lu Chen and former colleagues and coworkers after the Nobel Prize award ceremony at the Stockholm concert hall

I was a "doubting Thomas," soon to be refuted, because Südhof subsequently ploughed through the field of molecular neuroscience like a bulldozer. Using, for example, protein purification and protein-interaction screens, and inspired by genetic studies in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Drosophila melanogaster*, he discovered many dozens of synaptic vesicle proteins and other synapse components—and importantly, he invested early on in mouse genetic and electrophysiological approaches to study the function of the newly characterized proteins in neurons. Among many important discoveries, Südhof's lab showed that the SEC1 homolog Munc18 is a key regulator of the SNARE machinery (Hata et al., 1993), which controls SNARE complex formation and is absolutely required for synaptic vesicle fusion (Verhage et al., 2000) (Figure 1), and that the interplay between α-Synuclein and CSP controls SNARE protein stability and SNARE complex assembly (Chandra et al., 2005). Südhof highlighted these discoveries in his Nobel Lecture to indicate that the SNAREs alone cannot explain the intricacies of synaptic vesicle fusion, such as the presence of a primed and fusion-ready pool of vesicles at synapses, and that the dysfunction of SNAREs and other presynaptic components may play key roles in Parkinson's disease and other neurological and psychiatric disorders.

However, according to the Scientific Background provided by the Nobel Assembly, yet another discovery of the Südhof laboratory was at least of equal importance for awarding the 2013 Nobel Prize, namely the elucidation of the molecular mechanism by which Ca^{2+} ions trigger synaptic vesicle fusion. In 1990, Südhof determined the primary structure of Synaptotagmin-1 (Perin et al., 1990), a synaptic vesicle protein that had previously been identified by others, and showed that it contains a tandem of two C2 domains, which had first been found in Ca^{2+}-regulated PKC variants. These structural features of Synaptotagmin-1 indicated a possible role in the Ca^{2+} sensing step of synaptic vesicle fusion. Indeed, Südhof then demonstrated Ca^{2+} binding and Ca^{2+}-dependent phospholipid binding by Synaptotagmin-1 (Brose et al., 1992), found that Synaptotagmin-1 is required for fast synchronous neurotransmitter release (Geppert et al., 1994), and showed in a very elegant mouse genetic study by modifying the Ca^{2+} affinity of Synaptotagmin-1 in vivo that Synaptotagmin-1 really is the Ca^{2+} sensor of synaptic vesicle fusion (Fernández-Chacón et al., 2001). Subsequently, Südhof's group characterized many of the 16 mammalian Synaptotagmins and showed that only a subset of family members are involved in triggering Ca^{2+}-dependent synaptic vesicle fusion, with different characteristics. The exact molecular mechanism by which Synaptotagmins trigger vesicle fusion in a Ca^{2+}-stimulated manner has been a focus of fierce controversy for two decades. The current consensus is that both Ca^{2+}-dependent phospholipid binding and Ca^{2+}-dependent SNARE complex binding are involved.

Beyond Südhof's core work on the role of Synaptotagmins in Ca^{2+}-dependent synaptic vesicle fusion, the scientific justification of the 2013 Nobel Prize by the Nobel Assembly highlighted a final achievement of the Südhof lab, the discovery and functional characterization of Complexins (McMahon et al., 1995; Reim et al., 2001). This is particularly interesting because Südhof's view of Complexin function differs from that of his colaureate Rothman. While Rothman emphasized the "clamp" function of Complexin during his Nobel Lecture, Südhof interpreted his own work and that of several other neuroscientists to indicate that Complexin acts as an essential positive cofactor of Synaptotagmin that activates or "super-primes" SNARE complexes prior to fusion. It seems that some more model maturation is required in this regard, that this "wine" has still to age...

Like his colaureates, Südhof ended his Nobel Lecture with a discourse on a topic that had not been mentioned in the Scientific Background of the Nobel Prize provided by the Nobel Assembly, namely the problem of localized Ca^{2+} entry in presynaptic terminals. In most synapses studied so far, the synaptic vesicle fusion machinery is tightly coupled to voltage-gated calcium channels, which are the source of the Ca^{2+} trigger. However, the mechanisms by which such coupling might be brought about have long been unknown. Recent work by Südhof and colleagues has at least partly lifted this secret by demonstrating that the presynaptic scaffold protein RIM physically links the vesicle priming and release machinery to voltage-gated calcium channels (Kaeser et al., 2011). As with Schekman's and Rothman's Nobel Lectures, no such excursus

would have been necessary. The sheer number of Südhof's seminal contributions to our understanding of presynaptic function is unparalleled by any other scientist in the field, with his work on the Ca^{2+} regulation of transmitter release as the absolute highlight. There has been hardly any scientific meeting in the field of neuronal cell biology over the last decade where not a substantial fraction of talks and posters dealt with proteins that Südhof had first characterized—which is probably the best proof of the inspirational and visionary influence that Südhof's work has had in the neurosciences.

A Prize for Three?

In their Nobel Lectures, all three laureates emphasized the key contributions of their coworkers and collaborators, and in the literature citations here, I too have tried to honor their contributions. But students and postdocs come and go, whereas Schekman, Rothman, and Südhof had the genius and vision to pull off this amazing example of scientific discovery. It is therefore no question whatsoever that the 2013 Nobel Prize in Physiology or Medicine is extremely well deserved. As a neuroscientist and member of the "second generation" that followed the three pioneers, I am just a bit sad that there was no place for a few more laureates, so that Reinhard Jahn, Südhof's long-term ally, and Richard H. Scheller, a friendly competitor of Südhof and Jahn for two decades, could have also been honored. In this regard, cell biologists may feel similarly about Peter Novick and Tom Rapoport.

It is a difficult issue, and I do not envy the members of the Nobel Assembly that have to boil down a prize-worthy discovery to its essence. Unfortunately, only three can win, and because of this, the 2013 decision of the Nobel Assembly was smack on target. Much, much better, by the way, than the decision for the FIFA Ballon d'Or 2013, honoring the best footballer of the world. Regrettably, FIFA decided by vote of "experts" rather than after deliberations in a committee and elected Christiano Ronaldo, le beau, instead of the somewhat less beautiful but much more accomplished Franck Ribéry.

Chapeau bas Messieurs Schekman, Rothman, et Südhof—et Ribéry! Aah ... et Ronaldo!

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